Masses of Chassis

The tools of SynBio facilitate the design and application of genetic part and devices. This in turn allows biology to be programmed to perform functions of great and diverse benefit to society. Such functions include the sustainable development of medicine, agriculture, energy, materials and environmental remediation. Realisation of these goals is dependent on the use of suitable host organism or 'chassis' to implement these desired functions. Developments so far have been greatly focused on the use of model organisms.

Model organisms have been essential to furthering the understanding of biology across all kingdoms of life. Features such as amenability to lab culture and genetic manipulation, rapid life cycles, early discovery or sequencing and importance in human health have led research communities to sustain effort on study of species including *E. coli, Saccharomyces cerevisiae, Neurospora crassa, Drosophila melanogaster, Caenorhabditis elegans* and *Arabidopsis thaliana* amongst many others [1].

As model organisms, the gut bacterium *Escherichia coli* and baker's yeast *Saccharomyces cerevisiae* are attractive hosts for implementing genetic devices. They show relatively quick life cycles and heavy investment of resources has provided a plethora of tools, protocols and off-the-shelf kits for understanding, implementing and modelling genetic modification in these organisms [2, 3]. This historical focus placed on the 'workhorse' of molecular biology is reflected in The iGEM Registry of Standard Biological Parts, with the majority of available parts designed for use in *E. coli*.

As the widespread adoption of *E. coli* is largely based on legacy and convenience, rather than design rationale, the species may present many constraints and irrelevancies in relation to a function that it is tasked with. Many platform strains have been developed to overcome limitations in the industrial and biological capacity of wild-type *E. coli.* This includes implementation of N-linked glycosylation pathways[4] and block of endotoxin production [5]. Despite the demonstrated capacity to augment model organisms, there remains a pressing demand to explore and exploit the greater pool of natural diversity and the highly useful biological traits contained therein [6].

George Church recently presented a call for the field to move away from *E. coli* and adopt *Vibrio natriegens* as a keystone model for genetic engineering and rapid prototyping[7]. With generation times reported to be half that of *E. coli*, Lee et al. go as far as to state that the acceleration of iterative cloning steps allowed by this host will 'usher in a new era of advanced genomics' [7]. Although certainly an interesting prospect, the true benefit of adoption will be reliant on numerous factors including the biochemical nature of the host and the impact of this on the efficiency and cost of protocols. For example, the preference of *V. natriegens* for high salt conditions may affect DNA extraction [7, 8].

While high growth rate is beneficial in rapid prototyping, each disparate function will be optimally suited to a specific profile of traits such as media requirements, environmental conditions and innate metabolic tendencies. Due to relatively small

number, a current model organism is unlikely to be the optimal choice for implementing any given biochemical process. Model chassis may present some discord with the intended function of heterologous genetic components. To give an example, *E. coli* is not well suited to production of recombinant protein therapeutics, as it lacks the machinery required to instil eukaryotic post-translational modifications and shows disparate codon usage [9]. Modified *E. coli* strains as well as insect, yeast, plant and mammalian cell lines have been employed in biosynthetic and semisynthetic production of protein therapeutics. Each gives a unique profile of advantages and disadvantages including cost of substrates and equipment, production volume and scalability, product purity and profile [5, 10].

Native producer organisms and other non-model organisms may well show desirable qualities within the context of synthetic biology. The use of novel hosts could allow expansion of available chemical space[11], improvement of biosynthesis efficiencies[2] and creation of useful products from waste material [13]. Furthermore, availability of a variety of adaptable chassis is essential in expanding the capacity of synthetic biology to real world, out-of-lab applications [10]. Evolutionary pressure has outfitted species to cope with a great array of environmental conditions and exploitation of these natural adaptations may prove vital in furthering the reaches of biotechnology.

In recent demonstration of this potential, Choi et al. were able to demonstrate economical, low waste production of high-purity succinic acid from simple carbon sources through engineering a native producer, the rumen bacterium *Mannheimia succiniciproducens*[14].

Working with non-model organisms is non-trivial due to a lack of data, tools and characterised parts. For these reasons metabolic engineering in non-model species would previously have been considered to be a potentially exhaustive endeavour. Yet with the advance of technologies including next generation sequencing and CRISPR/Cas genome editing this is increasingly no longer true [6, 12, 15].

These technologies are increasingly accessible and are currently entering the hands of and empowering students and citizen scientists. The iGEM competition engages thousands of participants in developing parts and tools and such networked effort could help to bring species from obscurity to utility. A task that commercial science may shy away from due to no promise of or defined path to commercial reward.

Inspired by the need to diversify available chassis, our team has worked to develop some of the foundational tools and protocols required the for genetic perturbation in the non-model organisms *Penicillium roqueforti, Rhodococcus jostii and Synechocystis sp. PCC 6803.* By working with such a diverse collection of species, each possessing a range of interesting biochemical features of relevance to environmental, health and commercial interests, we hope to contribute to expanding the range of work that the iGEM Registry supports.

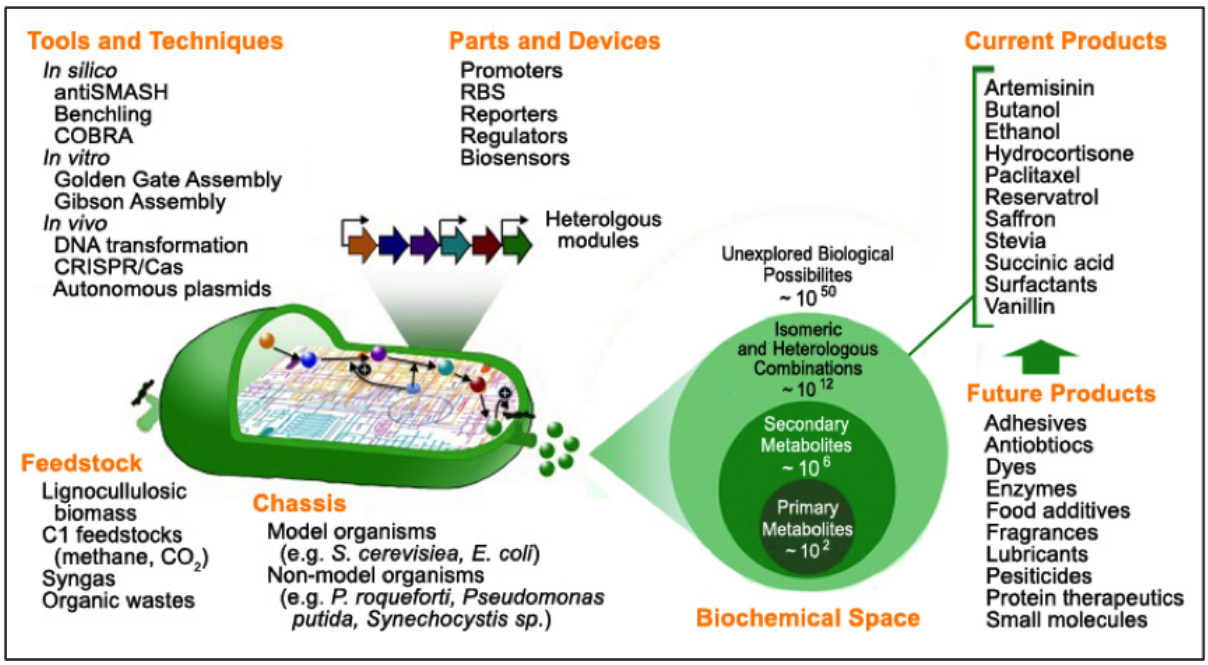

Fig. 1. Microbial biofactories. Adapted from Trinh and Mendoza (2016).

Microbial life can be engineered to perform useful functions including production of a huge array of important products. The development of tools and parts libraries gives a platform from which to accelerate genetic design and engineering. The exploitation of non-model organisms, both as a source of novel parts and as hosts of heterologous pathways, provides a viable path to greatly expanding the available biochemical space. Further, use of non-model organisms can extend the range of suitable feedstocks and production conditions, potentially yielding more sustainable production strategies.

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